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Review Article

Genomics of Vasculitis: Lessons from Mouse Models

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A genome analysis of mouse models may shed some light on the complex clinicopathological manifestations of systemic vasculitis. In the study of susceptibility loci to vasculitis in MRL mouse models, we found that systemic vasculitis developed through the cumulative effect of multiple gene loci, each of which by itself did not have a significant effect in inducing the related phenotype, thus indicating a polygenic system. The mice developed vasculitis in an additive manner with a hierarchical effect. Some of the susceptibility loci seemed to be common to those in other collagen diseases. Moreover, the loci controlling tissue specificity of vasculitis were present. One of the positional candidate genes for vasculitis showed an allelic polymorphism in the coding region, thus possibly causing a qualitative difference in its function. As a result, a particular combination of polygenes with such an allelic polymorphism may thus play a critical role in leading the cascade reaction to develop vasculitis, and also a regular variation of systemic vasculitis. This is designated as the polygene network in systemic vasculitis. (J Jpn Coll Angiol, 2009, 49: 11-16)

Keywords: collagen disease, genetic polymorphism, MRL mice, recombinant inbred strains, *Cd72*

Introduction

Systemic vasculitides are divided into primary and secondary types on the basis of their clinical features and pathological findings. The former includes vasculitis syndrome, which is regarded as a variation of collagen disease, and the latter includes vasculitides associated with other collagen diseases and those related to tumors, infections, and drug allergy. Vasculitis syndrome is clinically classified into various disease categories, but its actual clinical and pathological features are diverse, and not only its etiology but also pathogenesis is often unclear.

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Concerning genetic factors of vasculitis, susceptibility genes have been analyzed by case-control studies of individual vasculitis categories from deductive viewpoints based on the conventional inflammatology/immunology using the data about polymorphisms and SNPs of candidate genes, particularly, about primary vasculitis. Genes targeted in such studies were, first, those of MHC, followed by inflammatory cytokines, chemokines, growth factors, their receptors, lectins, adhesion molecules, Fcγ receptors, and NO synthetase. The biological basis of various vasculitides is expected to be clarified by comprehensive analysis of these individual data. However, the allelic and genomic polymorphisms of each gene are extremely diverse, show racial variation, and are associated with a wide variety of environmental factors. Therefore, there are many difficulties in clarifying the genomic mechanism and pathogenesis of vasculitis from such information.

In this report, the results of a series of our studies simulating the genomic mechanism of vasculitis using a vasculitis model mouse MRL/Mp-*lpr/lpr* (MRL/lpr) are presented. Conclusions that we reached were: Systemic vasculitis included in collagen disease is a disease that can be genetically differentiated from other lesions of collagen disease, it develops under the control of multiple

genes participating in an additive and hierarchical manner, and such genes can be understood as polygenes latently distributed in a population. The accumulation and combinations of susceptible alleles generated by genome crossing are considered to determine not only the occurrence and severity of vasculitis but also its tissue distribution and complication by other collagen diseases.

Vasculitis Model Mouse MRL/lpr

About 80% or more MRL/lpr mice develop systemic vasculitis at the age of 4–5 months. Vasculitis is remarkably observed in the arterial arches and interlobular arteries of the kidney, but it also occurs in small to medium-sized arteries of the pancreas, salivary glands, main aortic branches, tongue, and skeletal muscles of the limbs. Histopathologically, all lesions consist primarily of granulomatous arteritis. In its initial stage, systemic vasculitis begins with infiltration of CD4-positive lymphocytes around arteries but thereafter shows aggregates of activated macrophages and extends to destruction of the arterial external elastic laminae, degeneration of the media, and thickening of the intima.

This mouse was originally established by Murphy ED et al. of the Jackson Laboratory from an MRL strain of mice as a mutant mouse that develops systemic lymph node swelling and splenomegaly.[1] This strain exhibits lymph node enlargement (lymphoproliferation, hence designated as *lpr* gene) inherited in an autosomal recessive pattern at the age of 3–4 months due to accumulation of $CD4^{-}CD8^{-}B220^{+}$ T cells in lymph nodes. In addition, they spontaneously develop glomerulonephritis, arthritis, sialoadenitis, etc. as well as vasculitis. It also simultaneously exhibits various autoimmune phenomena. For this reason, this strain of mice has been studied widely as models of human polyarteritis nodosa, lupus nephritis, rheumatoid arthritis, and Sjögren syndrome.

Collagen Disease is Not Induced by Single-Gene Abnormality

When the MRL/lpr mouse was established, the series of manifestations of collagen disease was considered to be a single-gene disease due to the mutant gene *lpr* (a manifold effect of a single-gene mutation). In 1992, Nagata S' group clarified that this *lpr* gene is a deletion mutant of Fas, which induces apoptosis. This is due to insertion of a transposon into the second intron.[2] Fas molecules are expressed primarily in the activated T- and B-lymphocytes and macrophages in the immune system, and apoptosis of these cells is induced by binding of Fas ligand of activated T-lymphocytes. Therefore, Fas deletion mutation causes apoptotic insufficiency. This has been considered to lead to the appearance of autoreactive lymphocytes, prevent the control of immune responses and reactions, permit sustained activation of these cells and prolongation of inflammation, and cause autoimmune diseases.

However, an important problem is that collagen disease does not occur with *lpr* alone. At least C3H/lpr and B6/lpr mice, which are other mouse strains created by transduction of *lpr* gene, rarely develop collagen disease including vasculitis while they both show lymph node enlargement and exhibit various phenomena of autoimmunity such as increases in immune complexes, anti-DNA antibodies, and rheumatoid factors in the circulation and cytokine abnormalities.[3,4] Therefore, the phenotype of *lpr* only by itself is lymphoproliferation. On the other hand, MRL mice with no *lpr* gene manifest signs of collagen disease, though they are mild. Therefore, *lpr* gene can be understood as a "promoting factor" of collagen disease in MRL mice due to Fas-mediated apoptotic insufficiency, and the causative genes of collagen disease are considered to be background genes of MRL mice.[3]

This concept has recently been confirmed by the accumulation of individual clinicopathological data obtained by forced expression or deletion of immunity- and inflammation-related genes. Thus, pathological traits of autoimmune diseases expressed by manipulation of a single gene differ according to the genetic background of the individual. For example, of the IL-1Ra deficient mice, BALB/c mice develop arthritis, and B6 mice develop arteritis.[5,6] Among PD-1 deficient mice, B6 mice develop arthritis and glomerulonephritis, but BALB/c mice develop dilated cardiomyopathy, and the diabetic trait is promoted in NOD mice.[7–9]

Vasculitis Can Be Genetically Segregated From Nephritis and Other Collagen Diseases

On the analysis of pathological traits of mice produced by backcrossing between MRL/lpr mice and C3H/lpr mice, which do not develop collagen disease (N2 generation), or brother-sister inbreeding (F2 generation) to reconstitute the background genes, individual features of collagen disease appear separately. Thus, in these generations, some animals exhibit glomerulonephritis, vasculitis, sialoadenitis, and arthritis alone or in various

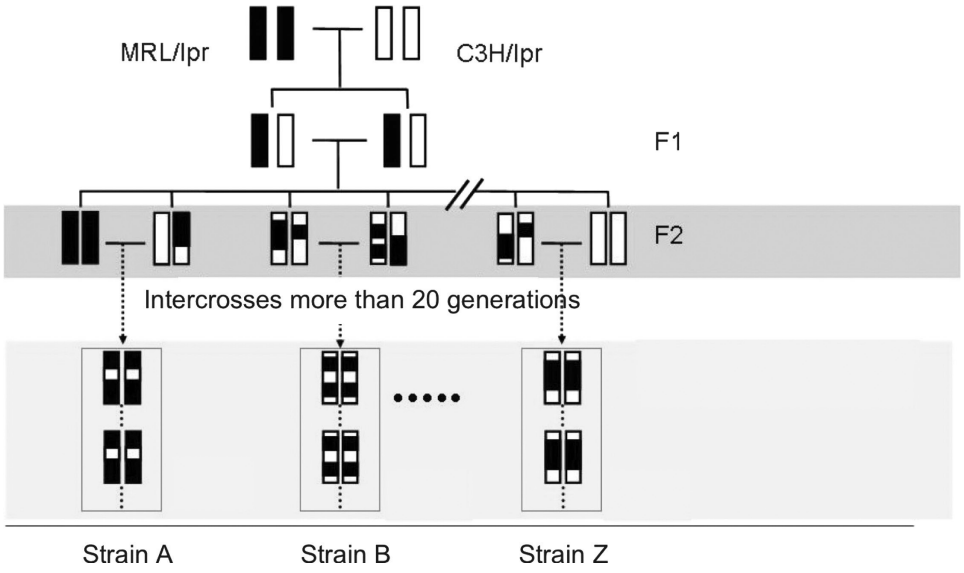

Fig. 1 Principle for the establishment of recombinant inbred (RI) strains of mice MXH/lpr. Genomic components of RI strains are composed of the genome of the parental strains, a collagen disease-prone strain MRL/lpr, and a resistant strain, C3H/lpr, in a mosaic form with homozygote.

RI strains	MRL	06	07	10	21	25	28	36	41	43	51	54	C3H
Arthritis	■		■	■		■		■					
Renal vasculitis	■	■		■				■	■				
Glomerulonephritis	■			■									
Dacryoadenitis	■								■				
IgG-RF	■					■	■		■	■			
MPO-ANCA	■			■	■	■			■	■			
Anti-dsDNA	■					■	■	■	■			■	

Fig. 2 Genetic dissociation of the pathological manifestations and autoantibodies of collagen disease among the recombinant inbred strains MXH/lpr.

combinations.[4] In addition, recombinant congenic McH5/lpr,[10] which rarely develops nephritis but frequently develops severer vasculitis, was established by histopathologically selective brother-sister inbreeding at each generation after the backcrossed N2 generation. This means that vasculitis-susceptible alleles were isolated and fixed. Indeed, in these mice, all vasculitis-susceptibility loci mentioned below are occupied by susceptible alleles.

Recently, we have established 15 MXH/lpr strains as recombinant inbred (RI) strains between MRL/lpr and C3H/lpr mice (**Fig. 1**).[11] In these strains, lines that develop glomerulonephritis, vasculitis, and arthritis randomly in various combinations were observed (**Fig. 2**). Moreover, autoantibodies that had been considered to be specific to particular pathological features were genetically dissociated in these RI strains, indicating that autoantibodies are not necessarily involved in the pathogenic mechanisms of various diseases. Therefore, the collagen disease traits of MRL/lpr mice are considered to be determined by genetically isolatable background genes specific to individual clinical and pathologic features. It has recently become possible to identify autoantibodies that increase concurrently with the occurrence of vasculitis by exhaustive search of protein antigens corresponding to the autoantibodies in these 15 strains.

This series of phenomena suggests that the traits of a single-gene mutant generally differ according to the specific genetic background of the mouse, and MRL/lpr mice

Table 1 Susceptibility loci to collagen disease identified by MRL/lpr mouse closses (revised, 2011)

Lesion	Symbol	Name	MGI : ID	Chr	Position (QTL)
Vasculitis	*Arvm1*	autoimmune renal vasculitis in MRL mice 1	MGI : 2149546	4	24.7 cM
	Arvm2	autoimmune renal vasculitis in MRL mice 2	MGI : 2149547	4	58.0 cM
	Arvm3	autoimmune renal vasculitis in MRL mice 3		3	55–61 cM
	Aaom1	autoimmune aortitis in MRL mice 1	MGI : 2680905	4	10.3 cM
	Aevm1	autoimmune extremity vasculitis in MRL mice 1	MGI : 2680906	8	34.4 cM
	Aevm2	autoimmune extremity vasculitis in MRL mice 2	MGI : 2680907	5	59.6 cM
Glomerulo-nephritis	*Agnm1*	autoimmune glomerulonephritis in MRL mice 1	MGI : 3582415	4	24.7 cM
	Agnm2	autoimmune glomerulonephritis in MRL mice 2	MGI : 3582416	4	45.8 cM
	Agnm3	autoimmune glomerulonephritis in MRL mice 3	MGI : 3582417	5	(56.0 cM)
Arthritis	*Paam1*	progression of autoimmune arthritis in MRL mice 1	MGI : 2387302	15	(17.8–18.7 cM)
	Paam2	progression of autoimmune arthritis in MRL mice 2	MGI : 2387303	19	(43–55 cM)
	Paam3	progression of autoimmune arthritis in MRL mice 3		7	24.5 cM
	Paam4	progression of autoimmune arthritis in MRL mice 4		2	45.0 cM
	Paam5	progression of autoimmune arthritis in MRL mice 5		1	100.0 cM
	Artmd1	arthropathy in MRL and DBA/1 mice 1	MGI : 3588383	10	37.2 cM
	Artmd2	arthropathy in MRL and DBA/1 mice 2	MGI : 3588384	3	27.7 cM
Sialoadenitis	*Asm1*	autoimmune sialoadenitis in MRL mice 1	MGI : 2150642	10	(38.4–40.0 cM)
	Asm2	autoimmune sialoadenitis in MRL mice 2	MGI : 2150643	4	(45.8–53.6 cM)
	Asm3	autoimmune sialoadenitis in MRL mice 3	MGI : 4410443	1	(69 cM)
	Asm4	autoimmune sialoadenitis in MRL mice 4	MGI : 4410444	2	(65.3 cM)
	Asm5	autoimmune sialoadenitis in MRL mice 5	MGI : 4410446	2	(82.1 cM)
Dacryoadenitis	*Adacm1*	autoimmune dacryoadenitis in MRL mice 1	MGI : 4410449	1	(64.1 cM)
	Adacm2	autoimmune dacryoadenitis in MRL mice 2	MGI : 4410450	2	(88.4 cM)
	Adacm3	autoimmune dacryoadenitis in MRL mice 3	MGI : 4410451	5	(63.9 cM)

MGI: Mouse Genome Informatics (http://www.informatics.jax.org/)

are considered to have indicated that many of the traits of knockout mice of various genes are not always specific to the genes.

Vasculitis is Caused By Polygenes

In MRL/lpr × (MRL/lpr × C3H/lpr) F1 and (MRL/lpr × C3H/lpr) F2 mice, QTL analysis was performed according to pathomorphological severity of each collagen disease, using polymorphic microsatellite markers between the two strains, and then the susceptibility loci of each pathological feature were mapped (**Table 1**). At least 3 loci related to the development of renal vasculitis were found. *Autoimmune renal vasculitis in MRL mice 1* (*Arvm1*) and *Arvm2* were mapped as recessive susceptibility loci for MRL alleles, and *Arvm3* located on *D3Mit42* as a recessive suppressive locus.[12)]

An additive effect was observed in the pathogenesis of vasculitis among these loci (**Fig. 3**). The incidence of vasculitis was 90% when all 3 loci were occupied by susceptible alleles but was only 48–60% when only 1 locus was occupied by a susceptible allele. In addition, the incidence was 79% when both *Arvm1* and *Arvm2* were occupied by susceptible alleles, but no additive effect was noted when *Arvm3* was combined with other loci, indicating the presence of hierarchy among these 3 loci.

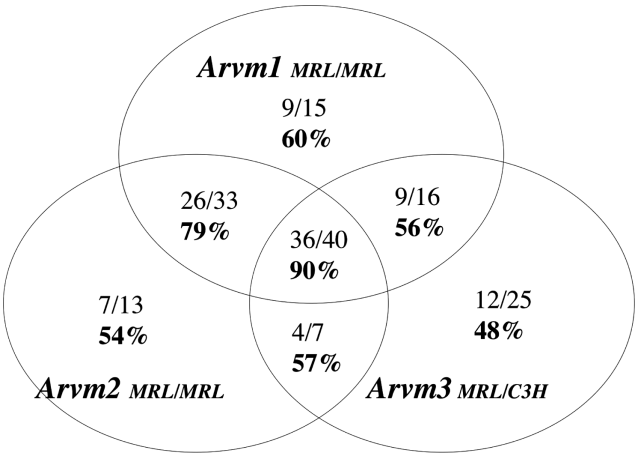

Fig. 3 Additive and hierarchical effects among the susceptibility loci to vasculitis in the kidneys. Combination of the loci with susceptible genotypes (*Arvm1; MRL/MRL, Arvm2; MRL/MRL, Arvm3; MRL/C3H*) regulates the incidence of vasculitis (%).

The Tissue Distribution of Vasculitis is Determined By the Genome

In MRL/lpr mice, granulomatous arteritis occurs in not only renal arteries but also main branches of the aorta and limb arteries. By analysis of susceptibility loci for vasculitis concerning various arteries, they were mapped at sites different from those related to the kidney

(**Table 1**).[13] A vasculitis susceptibility locus of major branches of the aorta [designated as *Autoimmune aortitis in MRL mice 1 (Aaom1)*] was located very close to *Arvm1*, a susceptibility locus of renal vasculitis on chromosome 4, but *Arvm2* and *Arvm3*, which are other susceptibility loci for renal vasculitis, showed no susceptibility. Also, the susceptibility loci for limb vasculitis *Autoimmuni extremity vasculitis 1 (Aevm1)* and *Aevm2* completely differed from *Aaom1* and were located on chromosome 8 and chromosome 5, respectively. This means the presence of genes that determine the tissue distribution of vasculitis.

Susceptibility Loci For Vasculitis and Glomerulonephritis Partly Overlap

QTL analysis in (MRL/lpr×C3H/lpr) F2 mice according to the morphological severity of glomerulonephritis suggested the presence of at least 3 loci related to the pathogenesis of glomerulonephritis (**Table 1**). We named them *Autoimmune glomerulonephritis in MRL mice 1* (*Agnm1*), *Agnm2* , and *Agnm3*.[14] They showed recessive susceptibility, semidominant susceptibility, and semidominant susceptibility, respectively, with MRL alleles and additive effects as in vasculitis.

Of these loci, *Agnm1* and *Agnm2* are nearly identical with, or very closely to, the vasculitis susceptibility loci *Arvm1* and *Arvm2*, respectively. Thus, the frequency of the concurrence of vasculitis and glomerulonephritis is considered to be high.

A Positional Candidate Gene For Vasculitis and Glomerulonephritis *Cd72*

Cd72 is located on *Arvm1*, a renal vasculitis susceptibility locus, and *Agnm1*, a glomerulonephritis susceptibility locus. It is a type II transmembrane penetration protein expressed primarily in B cells, has an immunoreceptor tyrosine-based inhibition motif (ITIM), which induces negative signals against cell differentiation and activation in the intracellular region, and proliferation and differentiation of B cells under antigen stimulation. mRNA of this gene was found to be polymorphic between MRL and C3H mice, involving insertion of 3 bases in exon 7 and deletion of 21 bases in exon 8 were noted in MRLmice.[12] This deletion in exon 8 was a result of aberrant splicing at the intron 7/exon 8 junction. *Cd72* of MRL mice retained the ITIM, but the marked mutations at least at 2 sites in the extracellular region mentioned above may cause functional differences of CD72.

Polygene Network of Vasculitis

Generally, genetic factors of diseases can be classified into 2 types. In one, a disease occurs as a result of induction of particular functional abnormality due to deletion or mutation of a single gene. In the other, mutation may be involved but does not induce a disease alone, and the involvement of multiple genes including complementary genes and gene polymorphism is required for the development of the disease. The biological concept of polygenic inheritance (Mather, 1949) applies to the second type (polygenic diseases). Therefore, while a single gene consistently induces particular clinical and pathological conditions in the former without being affected by any genetic background of the individual, clinical and pathological features in the latter are affected by the genetic background of the individual and are presented primarily as continuous quantitative traits. Also, the latter is reported to be susceptible to the effects of environmental factors, and diverse clinical and pathological features may be observed depending on the combination of polygenes. At least on the basis of the results of our analysis in MRL model mice, genetic factors of vasculitis belong to the latter, and the combination of polygenes (polygene network) produced by genome crossing is also considered to determine whether vasculitis is complicated by other collagen diseases and the diversity of the tissue distribution of vasculitic lesions.

Disclosure Statement

Masato Nose and the other authors have no conflict of interest.

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